AD_____

Award Number: DAMD17-00-1-0174

TITLE: Microsatellite and Chromosomal Instability in Breast Cancer

PRINCIPAL INVESTIGATOR: Svetlana Baranovskaya, Ph.D.

CONTRACTING ORGANIZATION: The Burnham Institute
La Jolla, California 92037

REPORT DATE: July 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | July 2004 | Annual Summary (1 July 2000 – 30 Jun 2004) |

**4. TITLE AND SUBTITLE**
Microsatellite and Chromosomal Instability in Breast Cancer

**5. FUNDING NUMBERS**
DAMD17-00-1-0174

**6. AUTHOR(S)**
Svetlana Baranovskaya, Ph.D

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
The Burnham Institute
La Jolla, California 92037

E-Mail: svetlana@burnham.org

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. Abstract** *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

During the reporting period, we analyzed a set of breast tumors for chromosomal and microsatellite instabilities, two fundamental pathways of genomic instability that play a critical role in the pathogenesis of several types of human cancers.

We found that 22% of all breast cancer cases have allelic imbalances in one or more of 14 microsatellite markers, which covered a region of 21MB of chromosome 7 around the EGFR gene. We found both amplifications (8%) and losses of heterozygosity (14%) of the EGFR-containing region of chromosome 7.

Microsatellite instability (MSI) was assessed with the use of 3 mononucleotide and 4 dinucleotide markers. None of the 202 samples analyzed showed any frameshift mutations in the mononucleotide repeats including polyA sequences in cancer susceptibility genes BRCA1 and BRCA2. One breast cancer tumor showed MSI at all four dinucleotide markers used for MSI status evaluation, but not at the mononucleotide markers. These data indicate that microsatellite instability is very uncommon (less than 0.5%) in breast cancer tumors. Our data show that MSI is not important in the pathogenesis or progression of breast cancer in contrast to other genetic mechanisms, notably recurrent chromosomal imbalances that dysregulate function of genes controlling cell growth, differentiation and apoptosis.

| 14. SUBJECT TERMS | 15. NUMBER OF PAGES |
|---|---|
| breast cancer, gene amplification, LOH, EGFR, MSI | 13 |
| | 16. PRICE CODE |

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

## Table of Contents

Inroduction.

Two major types of genetic aberrations have been found to play a critical role in tumor development: chromosomal segment deletions/amplifications (chromosomal instability) and point mutations, which often involve microsatellite sequences due to inactivation of the DNA mismatch repair machinery (microsatellite instability).

Chromosomal instability is found frequently in cancers and is believed to contribute to their development and progression through amplification of oncogenes or inactivation of tumor suppressor genes (reviewed in Gollin SM, 2004). The overexpression of the well known oncogene epidermal growth factor receptor (EGFR) is frequently detected in breast cancer (reviewed in Klijn JG, 1992). Chromosomal instability involving the EGFR containing locus of the chromosome 7p12 could be responsible for the EGFR regulation in breast cancer.

Microsatellite instability (MSI) is a distinct tumor phenotype that is being increasingly reported in a number of tumors including gastric, colon, endometrium and ovarian cancers (reviewed in Yamamoto H., 2002; Perucho M., 2003). The spectrum of the cancer genes that are targets for microsatellite instability may be cancer site specific. For example, BRCA1 and BRCA2 genes, which are found to be involved in breast cancer susceptibility, contain microsatellite repeats in their coding regions and, thus, may be targets for MSI in breast cancer. The prevalence of MSI in breast cancer, however, was not assessed.

During the reporting period, we estimated the relative involvement of the chromosomal and microsatellite instabilities in development of breast cancer.

Body.

*Evaluation of the chromosomal instability in breast cancer (tasks#1 and 3).*

We performed microallelotyping analysis of the 7p12 chromosomal region with 14 microsatellite markers, which covered a region of 21 Mbp in length. We also used distal telomeric markers D7S2477, D7S531, D7S2423 and D7S550 to determine if the whole chromosomal arm was gained or deleted in tumors. We employed multiplexed PCR to unambiguously distinguish chromosomal gains versus chromosomal losses (Fig. 1). The results of this analysis are summarized in Fig. 2.

This allelotyping analysis revealed two amplicons, 1,318 kbp and 2,202 kbp in length. The overlapping part of the two amplicons spanned 846 kbp and contained the EGFR gene. Two more cases (120, 108) showed allelic gain at several consecutive markers (including telomeric), which indicated a copy number gain of 7p for the first case and the entire chromosome 7 for the second one.

In addition to the gains of the 7p12 region, 14% of breast tumors demonstrated loss of heterozygosity (LOH). The lengths of deleted chromosomal segments ranged from 2.3MB (for example, case 112) to the entire chromosome (for example, cases 26 and 91). The smallest common region of these chromosomal losses contained the EGFR gene. Our data indicate that the 7p12 locus is not only amplified but also is frequently deleted in breast tumors and EGFR most likely is a target of these rearrangements.

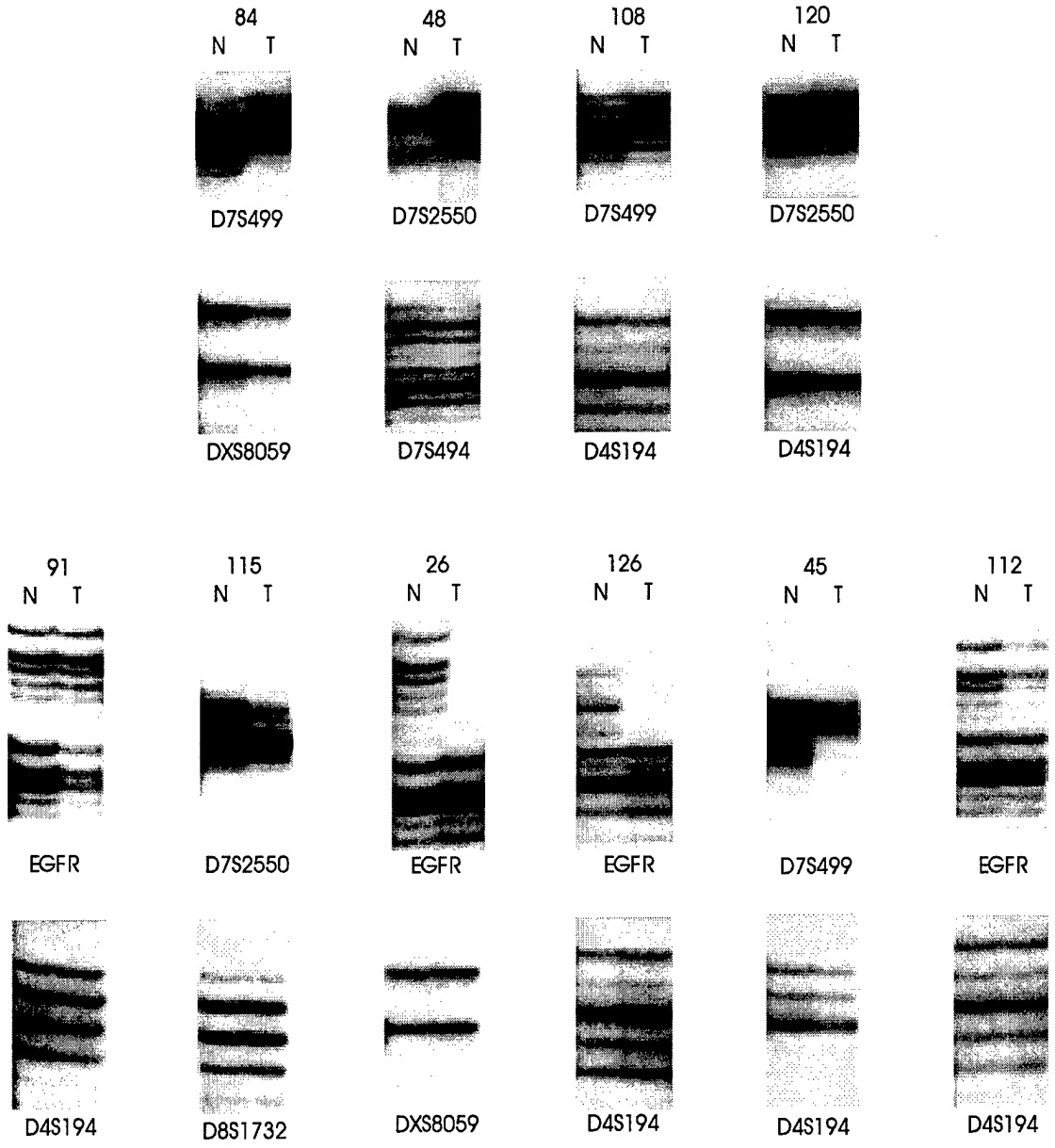


**Fig. 1**. Allelotyping of the breast tumors by multiplexed PCR. Top panel: microsatellite markers to be examined for gain/LOH. Bottom panel: microsatellite markers without allelic imbalances used as a reference (control). Names of the microsatellite markers analyzed are shown under the corresponding pictures. N, normal tissue; T, tumor tissue.
Gain at the D7S499 in cases 84 and 108; at the D72550 loci in cases 48 and 120.
LOH at the EGFR loci in cases 91, 26, 126 and 112; at the D72550 in case 115; at the D7499 in case 45.

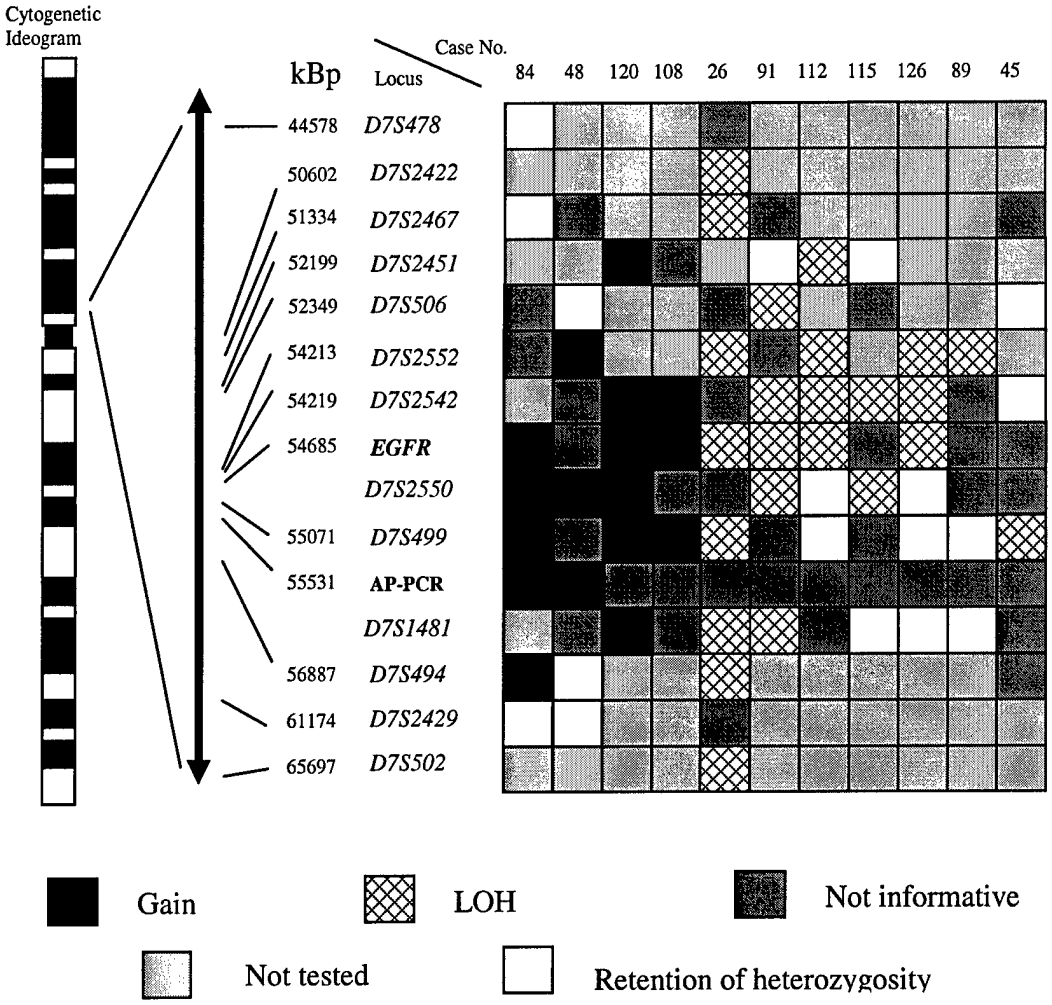


**Fig. 2.** Map of deletions and gains at 7p12 chromosomal region in a set of breast tumors.

No correlation was found between allelic imbalances and clinical parameters such as histological grade, presence of the metastasis, tumor size or tumor reccurence. The average age of breast cancer patients with AI at EGFR locus was less than the age of patients without AI (51 years versus 57 years). This difference however was not significant when a t-test used.

*Evaluation of the MSI in breast cancer (task#2).*

The set of 202 breast tumors was analyzed for the presence of microsatellite instability using three mononucleotide repeats (BAT-26, BRCA1, BRCA2) and four dinucleotide repeats (D5S123, D2S346, D7S2550, EGFR-CA). None of the samples showed a frameshift mutation in the mononucleotide repeats including polyA sequences

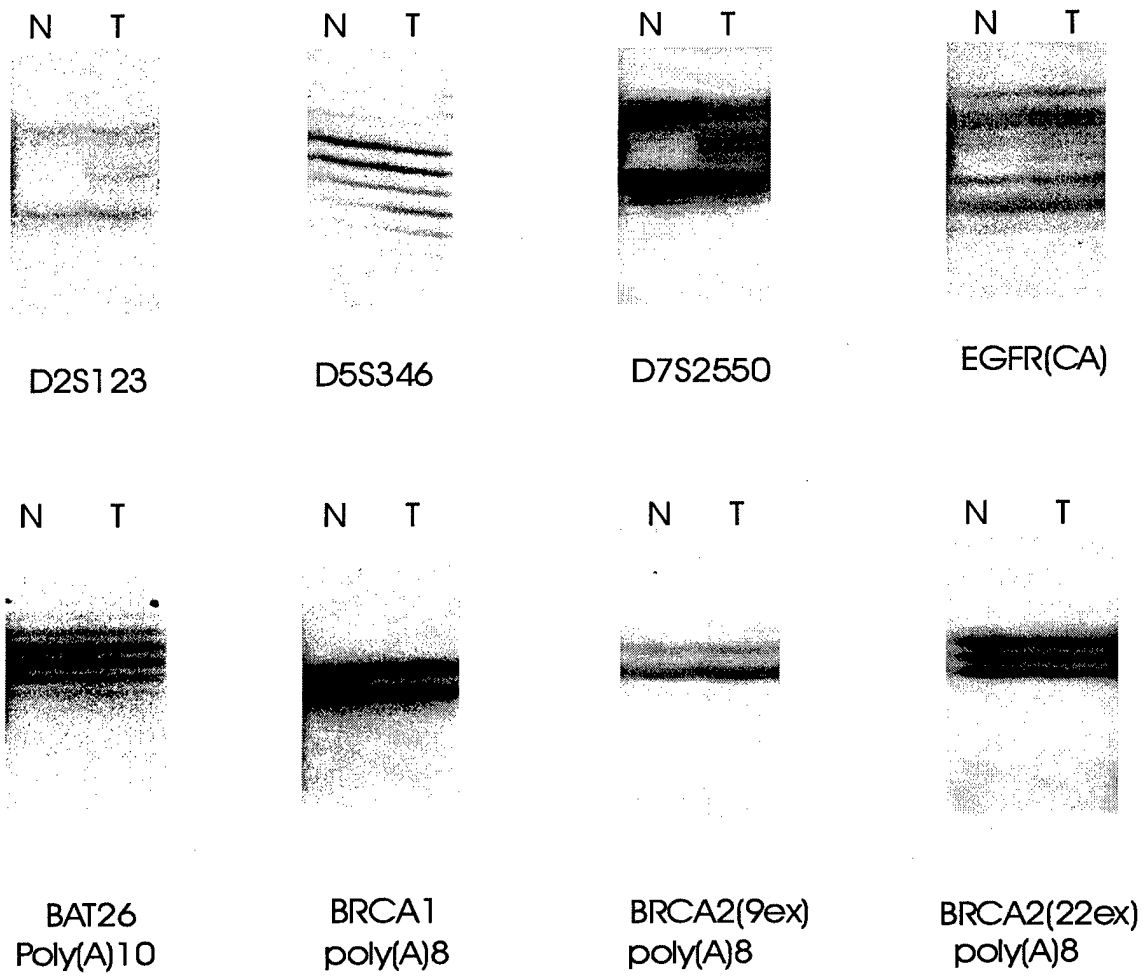


**Fig.3**. Microsatellite analysis of one breast cancer sample with MSI. Electrophoregrams of the repeat-containing PCR fragments amplified from normal and tumor tissue DNA isolated from MSI-positive breast cancer patient are presented. Top panel: Mutations in dinucleotide markers. Bottom panel: mononucleotide microsatellite markers without mutations. Names of the microsatellite markers analyzed are shown under the corresponding pictures. N, normal tissue; T, tumor tissue.

in the coding regions of cancer susceptibility genes BRCA1 and BRCA2. One breast cancer tumor showed MSI at all four dinucleotide markers used for MSI status evaluation but not at the mononucleotide markers (Fig.3).Our data convincingly demonstrated that microsatellite instability is very rare event in breast cancer tumors with a frequency of less than 0.5% of mutations in the dinucleotide repeats.

Key research accomplishments.

- We found that 22% of all breast cancer cases have allelic imbalances at least in one of the 14 microsatellite markes analysed. These results suggest that chromosomal instability is an frequent event in breast cancer.
- The new region of frequent allelic imbalances at 7p12 was identified and the EGFR gene most likely is a target of these rearragments. We showed that the EGFR locus is not only amplified in breast tumors, but it is also frequently deleted.
- Evaluation of the MSI status in the 202 samples showed that microsatellite instability is a very rare event in breast cancer tumors with a frequency of less than 0.5% of frameshift mutations in the dinucleotide repeats.

Reportable outcome.

Baranovskaya S, Malkhosyan SR. Frequent copy number alterations of chromosome 7p12 region in breast carcinomas (in preparation).
Baranovskaya S, Falchetti M, Perucho M, Malkhosyan SR. Modulation of EGFR expression in tumors of the microsatellite phenotype (in preparation).

Conclusions.

Our data demonstrated that microsatellite instability is not relevant for the development of breast cancer in contrast to other genetic mechanisms, recurrent chromosomal imbalances that lead to losses or amplification of the genes controlling tumorigenesis.

Our study has found also that change in the EGFR gene copy number is a frequent event in breast cancer. Both the amplification and the deletion of the EGFR gene facilitate tumorigenesis in a set of breast tumors. This finding has a high importance, because epidermal growth factor receptor is a target for some chemotherapy drugs.

References.

Gollin SM. Chromosomal instability. Curr Opin Oncol. 16(1):25-31 (2004).

Klijn JG, Berns PM, Schmitz PI, Foekens JA. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. Endocr Rev 13(1):3-17 (1992).

Perucho M. Tumors with microsatellite instability: many mutations, targets and paradoxes. Oncogene 22(15):2223-5 (2003).

Yamamoto H, Imai K, Perucho M. Gastrointestinal cancer of the microsatellite mutator phenotype pathway. Review. J Gastroenterol. 37(3):153-63. (2002).

# ASSISTANCE AGREEMENT

AWARD TYPE: [✓] GRANT (31 USC 6304) [ ] COOPERATIVE AGREEMENT (31 USC 6305) [ ] OTHER TRANSACTION (10 USC 2371)

| AWARD NO: DAMD17-00-1-0174<br>Modification P00002 | EFFECTIVE DATE<br>See Grants Officer<br>Signature Date Below | AWARD AMOUNT | Page 1 of 1<br><br>Danny L. Laspe<br>301-619-7147 |
|---|---|---|---|

**PROJECT TITLE:** Microsatellite and Chromosomal Instability in Breast Cancer

CFDA 12.420

| **PERFORMANCE PERIOD:** 1 July 2000 - 31 July 2003<br>(Research ends 30 June 2003) | **PRINCIPAL INVESTIGATOR:**<br>Svetlana Baranovskaya, Ph.D. |
|---|---|
| **AWARDED AND ADMINISTERED BY:**<br>U.S. Army Medical Research Acquisition Activity<br>ATTN: MCMR-AAA-A<br>820 Chandler St.<br>Fort Detrick Maryland 21702-5014 | **PAYMENTS WILL BE MADE BY:** EFT:T<br>ARMY VENDOR PAY<br>DFAS-SA/FPA (210) 527-8061<br>500 McCULLOUGH AVENUE<br>SAN ANTONIO, TX 78215-2100<br>*(SEE PARAGRAPH TITLED "PAYMENTS" FOR INSTRUCTIONS)* |
| **DUNS No:** 020520466 **TIN No:** | |
| **AWARDED TO:**<br>The Burnham Institute<br>10901 North Torrey Pines Road<br>La Jolla, CA 92037 | **REMIT PAYMENT TO:**<br>The Burnham Institute<br>10901 North Torrey Pines Road<br>La Jolla, CA 92037 |

**ACCOUNTING AND APPROPRIATION DATA:**

NO CHANGE

**SCOPE OF WORK:**

GRANT SCHEDULE

1. The basic grant Statement of Work is deleted. The revised 24 September 2002 Statement of Work submitted by the recipient is incorporated herein by reference.

2. The Principal Investigator is changed:

From: Izumi Nakamura, M.D.

To: Svetlana Baranovskaya, Ph.D

RECEIVED
NOV 6 2002
SPONSORED RESEARCH

| RECIPIENT | | GRANTS OFFICER | |
|---|---|---|---|
| **ACCEPTED BY:** The 24 September 2002 letter request signed by Jean Freiser is incorporated herein by reference as agreement.<br>**SIGNATURE** | | **UNITED STATES OF AMERICA**<br>Patricia A. Evans<br>**SIGNATURE** | |
| **NAME AND TITLE** | **DATE** | **NAME AND TITLE**<br>PATRICIA A. EVANS<br>GRANTS OFFICER | **DATE**<br>29 Oct 02 |

USAMRAA FORM 60-R, Feb 00

List of personnel receiving pay from the research effort:

**Svetlana Baranovskaya, PhD**

Publications:

**Baranovskaya S.**, Perucho M., Malkhosyan S.R. Comparative Hybridization of AP-PCR Arrays (CHAPA), new method for detection of single copy number changes in cancer cell genome (estimated date of submission October, 2004)

**Baranovskaya S.**, Falchetti M., Malkhosyan S.R. Modulation of EGFR expression by intron 1 dinucleotide repeat expansion in tumors of the microsatellite mutator phenotype (estimated date of submission September, 2004)

**Baranovskaya S.**, Malkhosyan S.R. Frequent copy number alterations of chromosome 7p12 region in breast carcinomas (estimated date of submission September, 2004)

**Baranovskaya, S.**, Soto, J.L., Perucho, M., Malkhosyan, S.R. Functional Significance of Concomitant Inactivation of the *hMLH1* and *hMSH6* Genes in Tumor Cells of the Microsatellite Mutator Phenotype. *Proc. Natl. Acad. Sci. USA* **98** (26): 15107-15112, 2001
The paper was chosen to be included in the "highlights of exciting advances from the primary literature" published by the *Nature Reviews* (Greenwood E. A perfect mismatch. *Nature Reviews* **2**:76-77, 2002)

Ohmiya, N., Matsumoto, S., Yamamoto, H., **Baranovskaya, S.**, Malkhosyan, S.R. and Perucho, M. Germline and somatic mutations in hMSH6 and hMSH3 in gastrointestinal cancers of the microsatellite mutator phenotype. *Gene,* **272**:301-313, 2001

Papayan L.P., Kobilyanskaja V.A, Sheydina A.M., **Baranovskaya S.S.**, Sirotkina O.V., Kargin V.D., Saltikova N.V., Belazo O.E., Golovina O.G., Papayan K.A., Tarkovskaya L.R. Changes in the system of hemostasis in patients with the hereditary thrombophilya. Therapeutic archive. 2000(7):47-51

Sirotkina O., **Baranovskaya S.**, Schwartz E. Molecular-genetic basis for inherited predisposition to venous thrombosis in Russian population. *Artherial Hypertention*, **5**:50-53, 1999

**Baranovskaya S.**, Kudinov S., Fomicheva E., Vasina V., Solovieva D., Khavinson V., Schwartz E. Age as a risk factor for myocardial infarction in Leiden mutation carriers. *Molecular Genetics and Metabolism,* **53**:155-157, 1998

Sverdlova A., Bubnova N., **Baranovskaya S.**, Vasina V., Avetisjan A., Schwartz E. Prevalence of the Methylenetetrahydrofolate Reductase (MTHFR) C677T mutation in varicose vein of lower limbs. *Molecular Genetics and Metabolism,* **64**:35-36, 1998

**Baranovskaya S.**, Shevtsov S., Maksimova S., Kuzmin A., Schwartz E. The mutations and VNTRs association in the phenylalanine hydroxylase gene of PKU patients in St. Petersburg. *J. Inher. Metab. Dis.* **19**:705, 1996

**Baranovskaya S.S.**, Shevtsov S.P., Maksimova S.P., Kuzmin A.I., Schwartz E.I. Spectrum of Phenylalanine Hydroxylase Gene Mutations in PKU patients in St. Petersburg population. *Proceeding of Russian Academy of Sci.*, V. **340**, N5, p.709-711, 1995

Eisensmith R.S., Goltsov A.A., O'Neill C., Tyfield L.A., Schwartz E.I., Kuzmin A.I., **Baranovskaya S.S.**, Tsukerman G.L., Treacy E., Scriver C.R., Guttler F., Eiken H.G., Guldberg P., Apold J., Svensson E., Naughten E., Cahalane S.F., Croke D.T., Cockbum F., Woo S.L.C. Recurrence of the R408W Mutation in the Phenylalanine Hydroxylase Locus in Europeans. *Am. J. Hum. Genet.* **56**: 278-286, 1995

Abstracts (selected)

**Baranovskaya S.**, Malkhosyan S. High throughput analysis of genomic aberration in cancer". The 4th Principal Investigator Meeting of the Innovative Molecular Analysis Technologies Program (June, 2003)

Malkhosyan S.R., **Baranovskaya S.**, Falchetti M., Li H.R., Pisarchuk K., Perucho M. Impact of mutations in non-coding microsatellite sequences on cancer gene expression. Proceeding of the AACR 44:1370, 2003

Malkhosyan, S.R., **Baranovskaya S.S.**, Perucho M. Functional Evidence for Secondary Mutator Mutations in Tumor Cells of the Microsatellite Phenotype. Proceeding of the AACR 42:828-829, 2001

**Baranovskaya, S.**,Piao Z., Malkhosyan, S.R. Comparative Hybridization of AP-PCR Arrays, New Method for Analysis of Losses and Gains of Genetic Material in Cancer Cell Genome.Proceeding of the AACR 42:746, 2001

Sirotkina O, **Baranovskaya S**, Volkova M, Sheidina A, Kobilianskaya V, Papayan K, Papayan L, Schwartz E, The prothrombin gene 20210GA variant and pulmonary embolism, *Eur. J. Hum. Genet.*, 8 (Suppl. 1): 80, 2000

Schwarz, E.I., Papayan, L.P., **Baranovskaya, S.S.**, Volkova, M.V., Sirotkina, O.V., Sheidina, A.M., Koblilianskaya, V.A., Kargin, V.D., Saltikova, N.B., Beliazo, O.E., Papyan, K.A., Golovina O.G. The genetics predisposition to thrombophilia in Russian population. *Thromb. Haemost.* Suppl.:385, 1999

Papayan, K.A., Cupatadze, D.D., Machin, U.U., Canina, L.I., Papayan, L.P., Sirotkina, O.V., **Baranovskaya, S.S.** Combined genetic pathology in the child with a thrombosis of an iliofemoral segment of the left lower extremity. *Thromb. Haemost.* Suppl.:385, 1999

**Baranovskaya S**, Sirotkina O, Kobilyanskaya V, Papayan L, Schwartz E. Unexpected results for distribution of 20210 G/A polymorphism in prothrombin gene in Russian population. *Eur. J. Hum. Genet.:* Suppl. (Geneva), 1999

Papayn, L; Kobilyanskaya, V; Kapustin; Blinov, M; **Baranovskaya, S**; Schwartz, E; Tarkovskaya, L; Beliazo, O; Khrolova, P; Kargin, V; Saltikova, N. Factor V Leiden as important risk factor of hypercoagulation and thrombophilia. *Int. J. Haemost. Thromb. Res.* 28: Suppl. 2, 1998

**Baranovskaya, S.**; Kudinov, S; Kobilyanskaya, V; Papayn, L; Vasina, V; Schwartz, E. High prevalence of Leiden mutation R506Q in patients with venous thrombosis in St.Petersburg, Russia. *Molecular Genetics* (Abstracts, p.126), 1998

Sverdlova-Sheidina, Anna; Bubnova, N; **Baranovskaya, S**; Vasina, V; Avetisjan, A; Schwartz, E. Prevalence of C677T mutation in MTHFR gene in patients varicose veins of lower limbs. *Molecular Genetics* (Abstracts:128), 1998

**Baranovskaya, S.**, Kudinov, S; Schwartz, E. The distribution of factor V Leiden mutation in three different ethnic population in the former USSR. *Thromb. Haemost.* Suppl.: 223, 1997

**Baranovskaya, S.,** Kudinov, S; Vasina, V; Fomicheva, E; Schwartz, E. Factor V Leiden mutation (Arg 506-Gln) among patients with myocardial infarction and control group. *Molecular Genetics* (Abstracts:142), 1997

**Baranovskaya S.**, Shevtsov S., Maksimova S., Kuzmin A., Schwartz, E. Association between mutations and VNTR in the phenylalanine hydroxilase gene of PKU patients in St. Petersburg. *The Society for the Study of Inborn Errors of Metabolism Proceedings* 09/12-15/1995

**Baranovskaya S.**, Shevtsov S., Maksimova S., Kuzmin A., Schwartz E. VNTR-haplotyping and mutation analysis of the phenlyalanine hydroxylase gene in St. Petersburg phenylketonuria families. *Eur J Hum Genet:* **4** Suppl. 1: 136-37, 1996

Schwartz, E; Shevtsov, S; Maksimova, S; Kuzmin, A; **Baranovskaya, S**. Spectrum of phenylalanin hydroxilase gene mutations in PKU patients in St.Petersburg. *Med.Genetik*, 2:164, (C19), 1995

**Baranovskaya, S.**; Shevtsov, S; Schwartz, E. A novel frameshift mutation in phenylalanin hydroxylase gene in two PKU probands of one Azerbaidjan family. *Med.. Genetik*, 2: 217 (H81), 1995

Kuzmin, A.I., Goltsov, A.A., Eisensmith, R.C., **Baranovskaya, S.S.**, Schwartz, E.I. and S.L.C. Woo. Molecular basis of phenylketonuria in seven populations from the Former Soviet Union. *Am. J. Hum. Genet.* **57**, Suppl.(4), 1995